Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

THE PRODUCTION OF CO_2 BY THE TYPHOID BACILLUS AND THE MECHANISM OF THE RUSSELL DOUBLE SUGAR TUBE

II. FERMENTATION OR RESPIRATION. PHENOL RED AS AN INDICATOR

WITH PLATE

HENRY J. NICHOLS AND CYRUS B. WOOD

From the Laboratory Division, Army Medical School, Washington, D. C.

In a previous article,[1] the production of CO_2 by the typhoid bacillus was attributed to fermentation or proteolysis, and in this sense it was stated that "this fact has apparently escaped previous observation." Our attention, however, has been called to the possibility that the CO_2 produced may be respiratory. The production of CO_2 by the typhoid bacillus as due to respiration is an old observation, dating back to Hesse,[2] in 1893. Hesse worked chiefly with glycerol agar. He estimated the O_2 used and CO_2 produced by a volumetric method after absorption with KOH and phosphorus. He tested a number of organisms, including the typhoid bacillus, and found that all use up O_2 and produce CO_2 by "breathing." The production of CO_2 in the case of the typhoid bacillus was most marked in the first three days. He advocated this method for testing the viability of a culture.

According to Gottschlich,[3] this finding was at first questioned, but has been fully confirmed by his own work and that of many others. Recently Novy[4] reached a similar conclusion that all bacteria, yeasts, molds and even protozoa produce CO_2 in their growth. Scheurlen[5] made the point long ago that the term "gas former" should be limited to gas bubble formers, as all organisms are really CO_2 producers. While in all this work it is assumed that CO_2 is respiratory, in the case of the visible gas formers it seems to have been tacitly assumed that the CO_2 is fermentative. Workers with the typhoid bacillus have apparently assumed, without sufficient reason, that the CO_2 of respira-

Received for publication Nov. 7, 1921.

---

[1] Nichols, H. J.: Jour. Infect. Dis., 1921, 29, p. 82.

[2] Ztschr. f. Hyg. u. Infectionskr., 1893, 15, p. 17.

[3] Kolle u. Wassermann, 1912, 1, p. 99.

[4] Personal communication.

[5] Internat. Beitrage y. Inn. Med., 1902, 2, p. 205.

tion need not be taken into account in dealing with fermentation reactions.

While there is, therefore, no doubt about the production of CO_2 by the typhoid bacillus, the question of its origin is a difficult one, involving as it does the whole subject of bacterial metabolism. This subject cannot be discussed to much advantage until more is known about the endo- and exo-enzymes of the typhoid bacillus. There are certain suggestions that the CO_2 is fermentative as well as respiratory. Acid production is visible in the butt of a Russell tube a few hours after inoculation and becomes marked after 24 hours, although the evidences of growth are slight. In other words, it seems as if an exoenzyme were present. More CO_2 is also produced in glucose than in plain mediums, although the growth is not materially different. On the other hand, the changes in a sugar-free Russell tube are the same as those in a regular tube, except in degree. The CO_2 in this case might come from proteolysis, but the fact that growth of the typhoid bacillus on any mediums produces CO_2 argues for an actual respiratory origin of some of the CO_2.

The process, whatever it is, is one in which CO_2 is produced on the slant. It escapes, leaving an accumulation of nonvolatile alkaline products. In the butt the same process occurs, but the CO_2 is retained by the medium and makes it acid. By degrees, however, the alkali from the slant diffuses downward and renders the whole tube alkaline.

As far as fermentation is concerned, formic acid may be taken as a typical example of the result of the first stages of decomposition of glucose. Under the influence of further growth, formic acid is apparently oxidized to H_2O and CO_2. Growth is, of course, favored by aerobic conditions and is more active on the slant than in the butt. Hence, the steps of fermentation are more complete on the slant than in the butt. In this sense the oxygen relations have some effect.

Phenol red has proved to be the best indicator to bring out the mechanism referred to, as it registers both on the acid and alkaline side. A pink phenol red Russell sugar tube, P_H 7.2-7.4, 24 hours after inoculation shows a yellow butt, P_H 6.8 and a red slant, P_H 7.8. In 48 hours the changes are more marked. Finally after a week, the whole tube becomes completely red from above downward. In tubes which are sealed, the butt and also the slant become and remain yellow or acid. The usual Andrade indicator shows the acid butt, but it is defective in showing an apparently unchanged slant. Litmus as an indicator shows the acid butt and also an alkaline slant, but the change is not clear cut. The reactions of phenol red are brilliant and convincing.

PREPARATION OF PHENOL RED RUSSELL DOUBLE SUGAR MEDIUM

Make extract agar (3% shred agar); clear; add 1% lactose and 0.1% glucose and 5% of a 0.02% watery solution of phenol red. Correct reaction to P_H of 7.2-7.4 hot. Tube, sterilize and slant so that there will be a deep butt and a long slant. The final reaction cold will be 7.2-7.4 as the tendency to a more alkaline reaction in cooling is overcome by a slight acidity developed in sterilization.

SUMMARY

The CO_2 produced by the typhoid bacillus in the Russell double sugar tube is probably fermentative as well as respiratory.

Phenol red is the best indicator as it shows both the acid change in the butt and the alkaline change in the slant.

The slant is not unchanged. It becomes alkaline and gradually the alkalis spread down and turn the butt alkaline.

PLATE 1

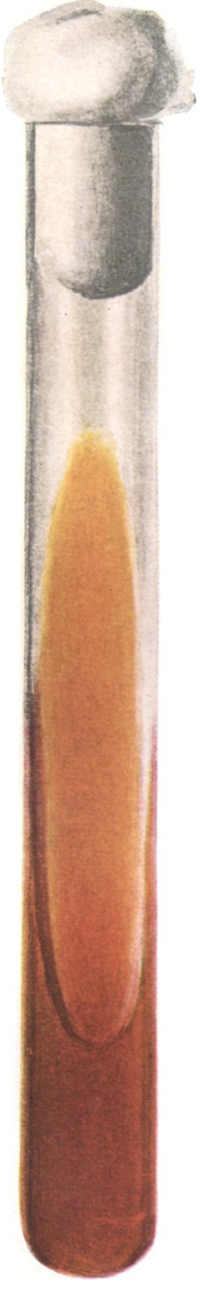

Figure 1

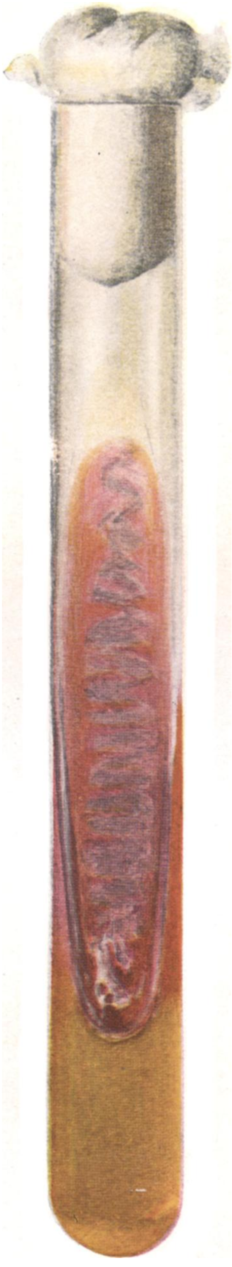

Figure 2

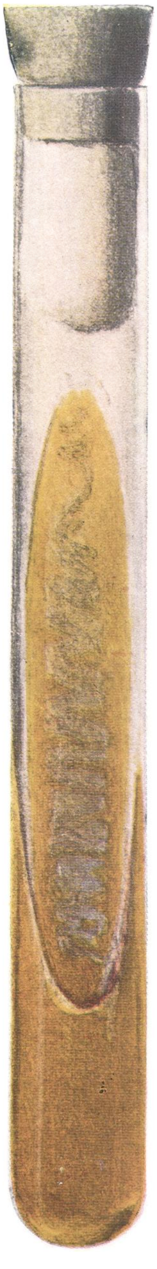

Figure 3

Fig. 1.—Control tube of double sugar when inoculated. Phenol red indicator, Ph about 7.4.

Fig. 2.—Double sugar tube inoculated with typhoid bacillus, "Rawlings" strain, after 48 hours. Ph. of butt, 6.6; Ph of slant, 7.8.

Fig. 3.—Double sugar tube inoculated with typhoid bacillus, "Rawlings" strain, 24 hours after inoculation with tube stoppered. Butt and slant have a Ph of 6.6.